Acquired Resistance to Antifolates by an Increase of γ-Glutamyl Hydrolase in H35 Rat Hepatoma Cells. *Rhee, M.S., Wang, Y., Yao, R. and Galivan, J.* Wadsworth Center for Laboatories & Research, New York State Dept of Health, Albany, NY 12201-0509.

Numerous cell lines have been developed with acquired resistance to antifolates. Established mechanisms of resistance are target enzyme amplification, target enzyme mutation leading to impaired drug binding, impaired transport, and impaired conversion to poly-γ-glutamates due to reduced transport or an impairment in folylpolyglutamate synthetase (FPGS). 5,10-Dideaza-tetrahydrofolate (DDATHF) inhibits *de novo* purine biosynthesis primarily by inhibiting glycinamideribonucleotide transformylase (GARTF) (1). It is transported by the reduced-folate carrier system and is active intracellularly as polyglutamate derivatives. H35 cells exposed to stepwise increases in DDATHF have become 80-fold resistant to the drug. The resulting cells are cross-resistant to methotrexate (MTX), 2-desamino-2-methyl-10-propargyl-5,8-dideazafolate (DMPDDF) and 10-propargyl-5,8-dideazafolate (PDDF) at the level of 55-, 60- and 12-fold, respectively. The resistant subline exhibits impaired transport for reduced-folate or MTX, which is reduced approximately 85 % compared to the parental cell line. The glutamylation of MTX in resistant cells is reduced by 95 %. FPGS activity is not significantly altered in the resistant cell extract, whereas γ-glutamyl hydrolase (γ-GH)activity is increased 7-fold. In addition, the amount of γ-GH secreted (2) by resistant cells is increased 4-fold. The enzyme from wild type and resistant cells both convert methotrexate polyglutamate (-Glu_n) directly to methotrexate while releasing poly-γ-glutamate (Glu_{n-1}), indicating the similarity and endo-peptidase nature of both enzymes. γ-GH is primarily lysosomal in its intracellular location with approximately 80 % of the total enzyme activity residing in this subcellular fraction in wild type and resistant cells. The combination of restricted transport and increased γ-GH can each contribute to the impaired glutamylation and the resulting resistance. Direct evidence for the contributory role of γ-GH in impaired polyglutamylation of antifolate comes from the result of study conducted with PDDF that enters cells independently of MTX/DMPDDF/DDATHF. PDDF is converted to its poly-glutamates in the resistant cells at 30 % of that in the wild type cells, which correlates directly with reduced sensitivity of the resistant cells to PDDF. The net effect of the increased γ-GH is a reduction in intracellular antifolylpoly-glutamates; hence a reduction in cytotoxicity of this class of drugs. This demonstrates for the first time that an elevated level of γ-GH is associated with acquired resistance to an antifolate. (Supported by NIH grants CA25933)

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